Kidney International, Vol. 5 (1974), p. 389–401

EDITORIAL

Diagnosis of immunopathologic renal disease

The participation of immune processes in the production of glomerular injury is generally accepted. The ability of the immunopathologist to readily identify and differentiate various immune pathways of injury has provided much needed insight into the mechanisms of glomerular injury and, in some cases, its etiology; it also has the potential of leading to "immunospecific" therapy. More and more, nephrology, transplantation and pathology programs are realizing the importance of immunopathologic classification of all renal disease. This is reflected in the ever increasing demand for immunopathologic services provided by groups like our own (Table 1). In this editorial we will explore the usefulness of some techniques available for detecting immune glomerular and tubular injury and see how the application of these techniques can help us identify, understand and follow the immunopathologic processes leading to renal damage.

In addition to antibody-initiated injury of the glomerulus, the recent identification of "nonimmune" complement (C) activation has caused considerable speculation regarding the roles of the properdin system, nephritic factors and microbial and parasitic products in the production of hypocomplementemia and glomerular injury mediated by mechanisms similar to those operating in immune injury. Since this will be the topic of a future editorial, this potential renal damage mechanism will not be dealt with in depth here.

Immunopathologic studies over the past 70 years, first in animals and now in man, have convincingly demonstrated two mechanisms by means of which antibodies can induce glomerular injury. In one, antibodies have specificity for antigens within the glomerular basement membrane (GBM) (so-called anti-GBM antibodies). In the other, antibodies react with circulating nonglomerular antigens and deposit passively as immune complexes in the glomerular filter. Either type of glomerular antibody deposition can result in tissue injury by bringing into play several interrelated mediator systems, including the C cascade, polymorphonuclear leukocytes and, potentially, such things as kinins, vasoactive amines and coagulation factors. These latter mediators may be of particular importance in certain anti-GBM nephritis patients who do not have glomerular C deposits (by immunofluorescence) and in experimental acute serum sickness in which C and polymorphonuclear leukocyte depletion prevents vasculitis but not glomerulonephritis. In addition to producing glomerular injury, antibodies may also react either directly with tubular basement membranes (TBM) in the form of anti-TBM antibodies or deposit in the TBM, peritubular capillaries and interstitium in the form of immune complexes, thereby providing the phlogogenic stimulus which may be responsible for the tubular and interstitial lesions

Table 1. Diagnostic renal immunofluorescence studies[a]

	Renal specimens submitted per year	Glomerular immunofluorescent deposits				
		Immunoglobulin pattern, %			Complement only	Negative
		Linear	Granular	Indeterminant	%	%
1970	396	7	49	5	8	31
1971	444	5	48	6	6	35
1972	456	4	48	2	12	34
1973	660	4	50	1	11	34
Total	1956					
Mean		5	49	3	9	34

[a] Tissue for these studies was submitted by nephrology and transplantation groups throughout the United States.

often observed in glomerulonephritis or interstitial nephritis.

Since both major antibody-induced mechanisms of glomerular damage can utilize similar mediators to effect injury, it is not surprising that, in general, routine clinical pathologic classifications are of little help in their differentiation. To further complicate matters, multiple histologic varieties of glomerular injury and their attendant clinical courses may be induced by a single immunologic stimulus in experimental models of glomerulonephritis such as those seen in chronic serum sickness in rabbits. A similar variability in glomerular histologic response is observed in the much more complicated immune complex type of glomerular injury which accompanies systemic lupus erythematosus (SLE). In this disease individual differences in antigen-antibody systems involved [1] might also contribute to the varied histologic response.

No light or electron microscopic features are pathognomonic of anti-GBM antibody-induced glomerular injury. Immune complex deposits, however, should be suspected in patients with increased mesangial regions thought by many to represent increased phagocytic activity. The subepithelial "spike" formation seen on silver stain in patients with membranous glomerulopathy results from extensions of basement membrane material (argyrophilic) between subepithelial immune complex deposits. At the electron microscopic level, electron dense deposits, presumed and actually shown to represent immune complex deposits, can be found in and about the GBM. For example, isolated subepithelial dense deposits or "humps" are seen frequently in acute poststreptococcal glomerulonephropathy and generalized electron dense deposits typify membranous glomerulopathy. In lupus nephritis, electron dense deposits are also frequently found in a subendothelial position, suggesting the particular antigen-antibody system involved may result in aggregates unable to pass through the GBM.

The availability of immunohistochemical methods (fluorescent and enzymatic) for detecting deposition of immunoglobulins (Ig) and other immunoreactive proteins within renal tissue has provided a relatively easy, available, and, we believe, necessary adjunct for the routine study of renal disease. These studies may be combined with elution of the antibodies [2] for *in vivo* or *in vitro* specificity studies. In addition to antibodies in glomerular deposits, circulating antibodies reactive with the GBM or nonglomerular antigens may be found. Evidence of cellular sensitivity can be sought and renal antigens in the urine detected, including those cross-reactive with the GBM. Growing interest in the possible role of nonimmune C activation in glomerular disease has led to the use of reagents to identify glomerular bound C components, properdin and related proteins, as well as to the search for factors in the circulation capable of activating the C cascade.

Immunofluorescence techniques. Immunofluorescence, the mainstay of immunopathologic diagnosis, depends on the use of fluorochrome (fluorescein isothiocyanate, tetramethyl rhodamine isothiocyanate, etc.) labeled antibodies for detecting Ig and associated phlogogenic proteins within renal biopsy specimens. Anti-GBM antibodies react with antigens distributed throughout the GBM and deposit evenly to produce a smooth, linear pattern, while immune complexes deposit at random within the glomerular filter to produce a granular or interrupted pattern. Experimental studies indicate that about 5 μg of diffuse linear IgG or 0.25 μg of discrete granular antigen/g of renal tissue can be detected by the immunofluorescence technique. The deposits may be further characterized by the class and subclass of immunoglobulin involved, and the presence of various C components and fibrinogen-related antigens (FRA) as well as nonglomerular immune complex antigens. Immunofluorescence techniques can also be utilized to identify cell types such as thymus-dependent lymphocytes, so-called T cells, within cellular infiltrates as seen, for example, in rejecting renal allografts [3].

Fluorescence is a property of a substance which, upon exposure to an exciting light of a proper wavelength, will emit a secondary light of a somewhat longer wavelength. By inserting a barrier filter in the optical path, the exciting light can be suppressed, allowing viewing of the secondary light. Fluorescein, for example, has a usable absorption maxima at 490 nm and emits a secondary light at 517 nm. The intensity of the light available for fluorescence study is dependent upon the light source and the filter combinations chosen. For maximal intensity the light is transmitted to the tissue via an immersed cardioid dark field condenser utilizing as few reflecting surfaces as possible. The recent introduction of vertical illuminators utilizing dichroic mirrors with selective, reflective properties has provided a useful alternate system, one which provides good illumination, particularly at high magnifications (the objective serving as its own condenser) [4]. Many investigators are enthusiastic about this new system and its freedom from an immersed dark field condenser; however, we do not feel that it provides as intense illumination as the transmitted systems (based on photographic exposure times). For routine diagnostic evaluation, either means of illumination is adequate.

High pressure mercury or xenon light sources pro-

duce the greatest light intensities. Recently, halogen-quartz light sources coupled with some of the interference filter systems have provided a much cheaper (although weaker) source of illumination. This latter system does not allow adequate utilization of other filter systems which are helpful in differentiating specific from nonspecific fluorescence. The recently introduced interference filters, which transmit wavelengths rather selectively from around 490 to 500 nm (optimal for fluorescein) or 546 nm (optimal for rhodamine), have resulted in greater intensities of fluorescence and greater ease in detection of antibodies labeled with these fluorochromes, either in tissue or on cell surfaces. Deposits scarcely visible with older, commonly used filter combinations are often quite bright when viewed with interference filters. Indeed, in a recent comparative study, granular glomerular IgM deposits were detected with interference filters in 95% of mice compared to only 16 to 48% of the same mice viewed with the older UG1 and BG12 exciter systems, respectively [5]. In our experience with fluorescein interference filters over the past four years, the differences have not been as striking as those found in studies in the mice; however, the infrequent viewer will be impressed with the light intensities produced by these interference filter systems and the ease with which deposits can be visualized. Because of the relative sensitivities of the various filter combinations used in routine renal immunofluorescence studies, their notation is imperative for meaningful interpretation of results.

No matter what fluorescence equipment and expertise is available for this use, the final result will be only as good as the reagents used and the quality of tissue available. Florid immunoreactive deposits can often be identified with less than optimal technique; however, for detection of less obvious deposits, the greatest care must be used. Heavy chain-specific antisera for detecting Ig classes and subclasses, which lack light chain reactivity, have largely replaced crude anti-gammaglobulin sera used in early immunofluorescence studies. Fluoresceinated anti-light chain sera are also useful in studies of conditions such as amyloidosis, in which glomerular and vascular deposits can sometimes be identified as being portions of an Ig light chain [6]. Any absorptions necessary to render the antiserum specific for the antigen in question should be done with insolubilized antigen, in order to prevent the inclusion of any soluble and potentially reactive immune complexes in the final reagent. A reagent which gives the greatest intensity of fluorescence with the least background staining is necessary and can be achieved most easily by utilizing fluoresceinated reagents which have undergone ion exchange chromatography to select a population of antibody molecules with an optimum fluorescein-to-protein ratio [7]. If one wishes to utilize commercial reagents, a satisfactory panel can be put together by screening a number of reagents from several companies, with emphasis on those purified by chromatographic techniques. Fluoresceinated-specific antibodies produced by elution from immunoabsorbants may also be used [8]. The specificity of the reagents must always be shown at the level of sensitivity of the immunofluorescence techniques by absorption with the specific antigen. Blocking with another unlabeled antibody of similar reactivity is also helpful. Reliance on gel diffusion analysis (a relatively insensitive technique) of the original antisera to determine specificity is foolhardy. Fluoresceinated antisera specific for "nonimmunologic" serum proteins (albumin, transferrin, etc.) should also be included in the panel of reagents so that nonimmunologic entrapment of serum proteins can be detected. Direct immunofluorescence techniques have sufficient sensitivity for routine renal studies, are simple to perform and require fewer controls than the more cumbersome indirect method.

Rapidly or "snap frozen" tissue (with liquid nitrogen) should be studied as soon as possible after procurement and the thinnest possible microtome sections obtained in a cryostat. Several different fixation procedures for the sections can be used, and it is important when a new technique is being tried that a variety of fixation methods as well as unfixed tissue be utilized to evaluate any untoward effects on the final result. The stained sections should be viewed in a standard way and attention paid to pattern and amount rather than intensity of fluorescence (a function of the reagent) of the deposit. The viewer should force himself to study all the structures in the section and not merely concentrate on glomeruli; tubular, interstitial and vascular deposits may be equally important. Serum proteins in tubular casts and in reabsorption droplets in tubular epithelium from patients with heavy proteinuria must be differentiated from immune deposits. Autofluorescent cellular granules, connective tissue and elastic lamina of vessels must not be confused with specific immunofluorescent deposits. Attention should be paid to any unusual autofluorescent material in the tissue; calcium deposits, for example, may exhibit a striking autofluorescence. Adequate specimens must be examined because presumed immue complex (granular) deposits may be quite "focal" and involve only a few glomeruli in a biopsy. We have occasionally seen biopsies with rather striking granular deposits in three or four of 50 glomeruli. On the other hand, granular deposits in membranous glomerulonephritis or linear deposits in

anti-GBM antibody induced nephritis are usually very uniform from glomerulus to glomerulus, so that a single glomerulus may be adequate for a tentative diagnosis.

Anti-GBM nephritis. The hallmark of anti-GBM antibodies is linear deposits of Ig, usually IgG, along the GBM [2]. Prior to severe destruction of the glomerular architecture, the deposit is very smooth and continuous; however,with advancing damage, the GBM becomes compressed, corrugated and fragmented so that continuous smooth linear deposits are no longer to be found. With experience, however, one can usually have a high degree of suspicion of the presence of anti-GBM antibodies even at late stages in the disease. The regular occurrence of focal or occasionally diffuse circumferential linear Ig deposits along the TBM is also extremely helpful in supporting a diagnosis of anti-GBM nephritis [2]. Occasionally, no Ig may be detected in the glomeruli available for study in biopsy specimens of patients with advanced anti-GBM nephritis; however, subsequent elution studies will demonstrate the presence of anti-GBM antibodies and so point out the value of this latter technique.

It is becoming increasingly clear that immunofluorescence detection of anti-GBM antibodies (linear staining) should be only the first step in making this diagnosis. Linear GBM deposits of Ig have been found in SLE [9] and often bright, but nonspecific (on elution), linear deposits of Ig (usually accompanied by albumin) have been found in the kidneys of diabetics [10], in about one-fourth of autopsy kidneys without renal disease, and not infrequently in kidneys after perfusion in preparation for transplantation [2]; all of which points out the necessity of corroborative elution studies or detection of circulating anti-GBM antibodies, or both, to support the diagnosis of anti-GBM nephritis. Certainly, the absolute necessity for a prerevascularization biopsy of a renal transplant for interpretation of any acute posttransplant Ig deposits is apparent.

In addition to the above conditions which may have "nonspecific" linear deposits, a significant number (about 10%) of renal biopsy specimens have a faint linear "accentuation" of the GBM when stained for IgG. This finding occurs most often in kidneys with the least histologic abnormality, so that tissue for elution studies is usually not available. Such patients do not have circulating anti-GBM antibodies and we feel that the staining merely represents an unexplained but detectable increase in the 2% IgG normally present in the GBM [11]. This finding may help explain such observations as the recently reported 13% incidence of linear staining in patients with usually benign hematuria [12]. We would stress, however, that these patients be observed in an appropriate manner until the significance of this type of staining is better understood.

The diagnosis of anti-GBM antibody-induced nephritis can be confirmed by demonstrating circulating anti-GBM antibodies. Indirect immunoflorescence utilizing normal human or primate kidney tissue as a target has been the standard test for such antibody determinations. This method detects antibodies in 87% of patients with anti-GBM antibody induced nephritis and pulmonary hemorrhage (Goodpasture's syndrome) and in about 60% of patients with anti-GBM antibody nephritis alone [2]. Hemagglutination techniques, although probably more sensitive, have a troublesome background of as yet unexplained reactivity in patients without other evidence of anti-GBM antibody nephritis [13, 14]. Gel diffusion is occasionally successful, but is the least sensitive of the methods available. The indirect immunofluorescence assay is limited by the ready availability of normal kidney target tissue and variable degrees of reactivity between targets. It does have the advantage of detecting other reactivities in serum, such as anti-TBM antibodies, antirenal tubular brush border antibodies and antinuclear antibodies.

We have recently extended our previous observations on the use of a radioimmunoassay for anti-GBM antibodies in renal eluates [11] to detecting circulating anti-GBM antibodies. Other groups are also working in this area [15]. To date, studies have been done on 356 patients with immunofluorescence characterization of their glomerular disease. The radioimmunoassay employs radiolabeled collagenase solubilized, and partially purified GBM, which is first reacted with the patient's serum. The mixture is then reacted with an anti-immunoglobulin antisera to precipitate the antibody bound antigen (C.B. Wilson, H. Marquardt and F. J. Dixon, in preparation). Sera from 22 patients with Goodpasture's syndrome and linear glomerular immunoglubulin deposits were reactive in the anti-GBM radioimmunoassay, including sera from two patients who had been only questionably positive by indirect immunofluorescence. The radioimmunoassay was also positive in 12 of 20 nephritic patients with linear staining who had no pulmonary involvement. In three of these patients the reactivity had been undetected by the indirect immunofluorescence assay. Neither assay was positive in the remaining eight patients, four of whom had elution-confirmed anti-GBM nephritis. With one exception, no diagnostic concentrations of circulating anti-GBM antibodies were found with either assay in 169 patients with granular (presumed immune complex) Ig deposits, including 13 with antinuclear antibodies. One patient

with granular immunoglobulin deposits had evidence of circulating anti-GBM antibodies by both radioimmunoassay and indirect immunofluorescence. This observation may be similar to that recently made by Klassen et al [16], on a patient who developed anti-GBM antibodies sometime after the onset of a classic immune complex type of membranous glomerulopathy. In addition, 19 nephritic patients with linear accentuation (IgG) of the GBM, 30 patients with glomerular C deposits alone and 96 patients with no immunofluorescent deposits failed to react in the radioimmunoassay.

The radioimmunoassay appears to be more sensitive than indirect immunofluorescence, being clearly positive in some cases when the latter is negative or equivocal. The reverse situation has not yet been observed. Direct comparison of the same positive serum sample shows that the radioimmunoassay is approximately four times more sensitive than immunofluorescence. The increased sensitivity should be helpful when patients are followed serially to determine when the anti-GBM antibodies disappear in preparation for transplantation.

Previous studies using the indirect immunofluorescence technique indicated that anti-GBM reactivity disappeared 3 to 25 months (mean, 8 months) after bilateral nephrectomy [2]. Too few observations of nonnephrectomized patients have been made to document whether nephrectomy is necessary for disappearance of circulating anti-GBM antibodies. While observations on autologous anti-GBM nephritis in sheep and early studies in man indicated that anti-GBM antibodies were more readily detected in nephrectomized subjects, further experience with patients, most of whom had advanced glomerular destruction, indicates that such kidneys have little immunoabsorptive capacity and circulating anti-GBM antibody concentrations are unaffected acutely by nephrectomy.

The fact that, to date, no sera positive by indirect immunofluorescence have failed to react in the radioimmunoassay with collagenase-solubilized GBM suggests that the reactivity of such antibodies resides in the roughly 25% of the GBM composed of noncollagenous glycoproteins. This finding would confirm previous absorption studies which indicated that positive serum reactions with indirect immunofluorescence technique could be absorbed with collagenase digested GBM [11]. Preliminary studies utilizing radiolabeled cartilage collagen do suggest, however, that occasional sera have reactivity with the collagenous portion of the GBM as well. This may help to explain why others have thought the disaccharide moiety of collagen was the nephritogenic antigen.

The radioimmunoassay has been automated and is now ready for large scale testing and screening of the nephritic population to determine the incidence of anti-GBM antibody-induced nephritis.

Various techniques to measure cellular sensitivity utilizing a patient's peripheral lymphocytes and GBM antigens may also be helpful in identifying immune responses to the GBM [13, 14, 17, 18]. There is little direct evidence, however, that sensitized cells play a role in the production of this form of glomerular injury, which can be readily produced by transferral of antibody alone.

Immunofluorescence studies suggest that anti-GBM antibodies are responsible for the glomerular injury in about 5% of the renal samples submitted to our laboratory (Table 1). The predominant Ig involved is IgG, with IgA and IgM only infrequently being identified [2]. IgG subclasses have also been studied [19, 20]. Fibrinogen-related antigen is often quite prominent in areas of crescent formation seen in the rapidly progressive form of the disease, but little FRA is usually present within glomerular capillary loops. Little is known about the events responsible for the induction of the anti-GBM response; however, a careful evaluation of toxic exposure [21] or viral infection [22] is mandatory. Experimental demonstrations that endogenous basement membrane-like materials from the urine can induce "autoimmune" anti-GBM nephritis if properly presented suggest the possibility that the inciting event might also be endogenous.

In patients with anti-GBM antibody-induced glomerulonephritis and pulmonary hemorrhage (Goodpasture's syndrome) linear Ig deposits can often be identified binding to the alveolar basement membrane; in addition, antibodies capable of reacting with the GBM have been eluted from the lungs of these patients. These observations as well as the induction of anti-GBM antibodies with lung tissue in experimental animals suggest a common mediation mechanism. Patients with immune complex glomerulonephritis occasionally have pulmonary hemorrhage so that immunopathologic classification of Goodpasture's syndrome is mandatory [23, 24]. Anti-GBM antibody-induced glomerulonephritis and pulmonary hemorrhage may present with renal abnormalities, lung abnormalities or a combination of both [2]. There is little clear evidence about which organ system is primarily involved. It is well recognized that anti-GBM antibody-induced nephritis may occur without pulmonary disease. On the other hand, we have recently observed a patient with pulmonary infiltrates, hemoptysis and anemia who was diagnosed clinically as having idiopathic pulmonary hemosiderosis. This patient had linear deposits of IgG bound along the

alveolar basement membrane and evidence of circulating anti-GBM antibodies. He has had no clinical evidence of renal involvement, although a kidney biopsy six months after his initial episode revealed linear deposits of Ig along the GBM.

Immune complex nephritis. Granular deposits of Ig usually accompanied by C are the hallmark of experimental and spontaneous immune complex glomerulonephritis in animals as well as in an ever increasing number of examples in humans (Table 2). It is thought that most of the deposits originate as circulating immune complexes; however, it is increasingly apparent from experimental studies that glomerular bound immune complexes are in dynamic equilibrium with the circulation and antibody or antigen, or both, can combine with or be dissociated from them. It is also not unreasonable to suspect that Ig-Ig aggregates (often cryoglobulins) and possibly C aggregates of the proper configuration could lodge in glomeruli in much the same way as we presume immune complexes do. Since *in situ* formation of immune complexes is thought to be involved in experimental thyroiditis in mice and in renal tubular lesions of rabbits injected with tubular antigens, a similar phenomenon might occur in glomeruli as well. For example, recent experimental studies have demonstrated clearly that antibody can react with material localized in the mesangium [25].

Care must be exercised in examining human material since granular deposition is often focal and may not involve all glomeruli. It is also difficult to be certain of the "specificity" of irregular or granular deposits of Ig and C in glomeruli that also have large amounts of FRA. If localization of the immunoreacting deposition conforms to that of the FRA, it is probable that serum proteins are merely entrapped in the fibrin coagulum, as suggested by the presence of albumin and other "nonimmunologic" proteins.

Granular deposits of Ig usually accompanied by complement are present in about one-half of the renal biopsy specimens in our ongoing study (Table 1). If from this group are selected those patients thought on historic and clinico-pathologic grounds to have glomerulonephritis (many of whom are far advanced), then the incidence of granular deposition increases to between 70 and 80%. A generalized summary of the type of deposits found in various clinico-pathologic varieties of glomerulonephritis is presented in Table 3. The pattern of deposition may be intense and generalized corresponding to the subepithelial dense deposits visualized by electron microscopy in membranous (or extramembranous) glomerulopathy, or scant and involving only segments of the GBM or mesangium in more focal glomerular lesions. Occasionally a patient with fulminant glomerulonephritis may have little or no detectable deposits within the glomeruli. Whether this

Table 2. Human immune complex glomerulonephritis

A. Immune complex glomerulonephritides in which antigen, specific antibody or both have been identified in granular glomerular immunoglobulin deposits	
Exogenous antigens	
Foreign serum proteins, drugs	Serum sickness
Streptococcal antigens	Poststreptococcal glomerulonephritis
Staphylococcal antigens	Infected venticuloatrial shunts
Plasmodium malarial antigens	Quartan malarial nephrosis
Enterococcus	Subacute bacterial endocarditis
Australia antigen	Hepatitis
Oncornavirus-related antigen	Lymphoma-leukemia
Measles antigen	Subacute sclerosing panencephalitis
Endogenous antigens	
Nuclear antigens	Systemic lupus erythematosus
Thyroglobulin	Thyroiditis
Renal tubular antigens	Three cases of membranous glomerulonephritis in Japan, sickle cell anemia
Carcinoembryonic antigen	Colonic carcinoma
? Ig	Cryoglobulinemia
B. Conditions with presumed immune complex glomerulonephritis (granular glomerular immunoglobulin deposits) in which antigen-antibody systems should be identifiable	
Drugs	Penicillamine, gold, heroin, prophylactic inoculations
Infections	Bacterial endocarditis, leprosy, syphilis, schistosomiasis, filariasis, infectious mononucleosis, varicella, Landry-Guillain-Barré-Strohl syndrome
Neoplasia	
Sarcoidosis	

Table 3. A summary of glomerular Ig, C3 and FRA deposits in patients with glomerulonephritis (GN)[a]

	Glomerular immunofluorescence
A. Anti-GBM GN	
Goodpasture's syndrome and rapidly progressive GN	Linear IgG, infrequent IgA, IgM. C3, if present, usually irregular. FRA prominent in crescents.
B. Presumed immune complex GN	
Proliferative GN, including poststreptococcal	Scattered granular IgG. Variable presence of IgA and IgM. C3 usually present and may be observed in the absence of Ig. FRA usually absent.
Rapidly progressive GN	Granular IgG, variable IgA, IgM. C3 usually present. FRA prominent in crescents.
Membranous GN	Generalized discrete granular IgG, C3, with variable IgA, IgM. FRA usually absent.
Membranoproliferative GN	Coarse peripheral C3 usually accompanied by IgG, and often IgA and IgM. FRA deposits variable.
Focal GN, including mesangial hypertrophy	Segmental granular and mesangial IgG, C3 often with prominent mesangial IgA. IgM and FRA variable.
Focal sclerosis	Segmental granular IgG, IgM, C3. IgA and FRA usually absent
Systemic lupus erythematosus	Prominent generalized or segmental granular and mesangial IgG, IgA, IgM and C3 deposits. FRA variable.
Henoch-Schonlein purpura	Prominent segmental and mesangial IgG, IgA, C3 and FRA. IgM variable.
C. Lipoid nephrosis	
Minimal change lesion	No IgG, IgA, IgM, C3, FRA deposits.

[a] Useful references: 2, 26–33, 67, 69, 86–91. GBM, glomerular basement membrane; FRA, fibrinogen-related antigens

represents a loss of previously deposited material due to the intensity of the inflammatory response, or another as yet unidentified mechanism of glomerular injury, is unknown. Kidneys thought to represent end stages of glomerulonephritis may be free of Ig deposits or if the deposits are present they may be limited to the least damaged glomeruli. The few serial studies available suggest that Ig deposits may disappear as glomerular damage advances, while C deposits may remain or even intensify. It is of great interest that, in our experience, roughly 10% of nephritic kidneys have granular deposits of C alone (Table 1). The possible significance of this finding will be discussed later.

In addition to the distribution of the deposits, the class or subclass of Ig may be identified by using monospecific antisera. For example, glomerular IgE deposits were recently reported in patients with lipoid nephrosis; however, the finding has not been subsequently confirmed [26, 27]. Granular mesangial deposits of IgA, as the predominant immunoglobulin, have been identified in Henoch-Schonlein purpura [28, 29], as well as in patients with recurrent and often benign hematuria [30–32]. Although the IgA deposits usually contain IgG and C (and involve the GBM as well in Henoch-Schonlein purpura), their predominance and localization has generated great interest. The granular nature of the deposits would suggest their origin as immune complexes and their localization predominantly in the mesangium would suggest that they were relatively large and insoluble, thus leading to a mesangial localization and relatively benign clinical pathology. Binding of IgA to bacterial products lodged in the mesangium could also be considered and should be determined. Granular IgA GBM deposits may also be diffuse and are frequently present in patients with SLE [33].

Based on findings in animals (serum sickness, viral infections, etc.) and on an ever increasing number of human examples (Table 2) most, if not all, kidneys containing granular deposits of Ig are presumed to have immune complex deposits. It goes without saying that intensified efforts must be made to identify the antigen-antibody systems in each patient. If sufficient tissue is available, the immune complexes may be removed for study by elution in buffers known to dissociate antigen-antibody bonds. The eluted materials can then be studied by a variety of immunologic and physical chemical techniques in order to define the nature of the antigen and the antibody specificity. In experimental serum sickness in which radiolabeled antigen or antibody is used, it is possible to easily elute about 70% of the bound immune complex material; however, if no attempt is made to separate the antigen and antibody while still dissociated, upon return to

physiological conditions they will recombine (70%) and probably be lost to further study. Since the antigen is usually unknown, multiple methods may need to be tried before a satisfactory separation is accomplished. In addition to separating the antigen and antibody, destruction of the antigen (or antibody), as in the case of DNA with DNAase, would also reduce recombination.

If only biopsy specimens are available, immune-complexed antigen may be sought using immunofluorescence. Since antibody from the circulation may have covered the antigenic portion of the immune complex, partial elution may be required to uncover sufficient antigenic sites for detection with the fluoresceinated antiserum. Glomerular immune-complexed antibody may be similarly sought using fluoresceinated or radiolabeled antigen.

Detection of similar antigen-antibody complexes in the circulation would establish their nonrenal origin and has been undertaken successfully in animals with serum sickness and viral nephritis, as well as being suggested by serial fluctuations of circulating DNA and anti-DNA in humans with SLE. Cryoglobulins in patients with nephritis may also be a relatively easily isolated serum fraction in which to detect immune complexes. Sensitive and clinically applicable methods of detecting circulating immune complexes have been slow in coming. Sera with decreased complement concentrations or anticomplementary activity should be suspect. Analytical and sucrose density ultracentrifugation, gel filtration and C1q and monoclonal rheumatoid factor precipitation are of varying sensitivity and reactivity, and serve primarily as research tools. Platelet aggregation, although perhaps more sensitive, is plagued by nonreproducible platelet preparations. Two methods under current investigation, complement consumption utilizing minute and limiting quantities of complement [34] and binding of complexes to either Ig or C receptors on cultured lymphocytes, show promise [35]. If such techniques were to be successful, they would play as prominent a role in diagnosis of nephritis as does detection of anti-GBM antibodies. They would also be helpful in determining optimum timing for renal transplantation in an attempt to avoid recurrent immune complex glomerulonephritis.

The patient's physician must serve as a detective, narrowing the field of potential antigen-antibody systems which could act either singly or in combination to produce the numerous, as yet "idiopathic," forms of glomerulonephritis with granular glomerular Ig deposits (presumed immune complexes). A careful history and clinical evaluation should cover possible antigen exposure, including drugs and inoculation; associated microbial infections, including persistent viral infections, such as hepatitis; renal trauma and vascular insult or toxic exposure; neoplasia; and associated autoimmune responses, such as thyroiditis or SLE. Since chronic viral infections are nearly universally associated with immune complex glomerulonephritis in animals, viral infections would be suspect in man as well. Attempts to measure circulating antiviral antibody responses have not resulted in detection of common agents; however, isolated and unexplained elevations in antibody response have been seen, especially in patients with SLE [36]. Attempts to isolate viral agents from renal biopsy tissue have been disappointing [37] and, even if successful, the isolate would be difficult to relate to immune complexes. Virus-like particles in endothelial cells so common in SLE are now being found with increasing frequency in other conditions [38] and may only reflect degenerative changes within the cell. If circulating antigen and antibody were present in nearly equivalent proportions, they would combine and neither would be detectable by the usual immunologic techniques, a common situation in viral nephritis in animals. It may then be profitable to utilize other measures of immunity, such as cellular sensitivity, to detect otherwise "masked" antibody responses as a lead to antigen-antibody systems involved in a particular case. Table 2 lists a number of presumed immune complex glomerulonephritides in which antigen identification should be relatively easy. Also included are conditions such as sarcoidosis and neoplasia in which antigen identification could result in an understanding of the etiology of these otherwise obscure conditions.

Immune complexes may induce arterial as well as glomerular injury as, for example, in SLE. Hepatitis SH antigen-antibody complexes have been found in vascular lesions of some polyarteritis patients [39, 40] and may produce glomerulonephritis as well [41]. Immunofluorescence studies of glomeruli of patients with Wegener's granulomatosis [42] and some patients with scleroderma [43] suggest that immune complexes may be implicated in these conditions as well. Vascular deposits of Ig, C and FRA have been found in patients with malignant hypertension ([44, 45], C. B. Wilson, unpublished observations). It is not known if such deposits have immunologic specificity or are merely trapped in areas of inflammation; findings of elution studies would be of particular interest. Absence of Ig and C in renal problems associated with consumptive coagulopathy in which FRA are prominent, such as the hemolytic uremic syndrome [46, 47], suggests that immune reactions of the types here discussed are probably not involved. Likewise, immunopathologic studies of the pathogenesis of familial nephropathy

have not shown that either anti-GBM antibodies or immune complexes are involved [48, 49]. Mauer and associates have recently observed that Ig and C deposits accumulate in glomeruli of rats with experimental diabetes mellitus [50, 51]. These findings should renew interest in immunopathologic studies of the renal involvement found with this disease in man.

Complement and glomerular disease. Depressions in serum C have been used to implicate immune processes in the production of glomerular disease. Indeed, hemolytic C (CH50) is often depressed in acute poststreptococcal glomerulonephritis and SLE; however, in many other presumed immune complex types of glomerulonephritis, as well as in anti-GBM antibody induced nephritis, serum C concentrations are often normal [52]. The recent recognition of alternate C activation, beginning at C3 and bypassing the classical C1-4-2 reaction, has emphasized the need to detect both early and late acting C components in the serum [53, 54]. The alternate pathway, which involves the interaction of an as yet incompletely defined series of proteins, including properdin [55], C3 proactivator [56, 57] and the C3b fragment of C3 [58, 59], may be activated by immunoglobulin, including IgA and nonimmune means with bacterial lipopolysaccharides, yeast (zymosan) and particulate inulin. Factors are present in the sera of some hypocomplementemic patients which can activate this pathway [60]. Such factors are detectable by observing their ability to activate C3 in normal serum (detected by changes in electrophoretic mobility) or to lyse glutathione sensitized red blood cells (C. M. Arroyave, E. H. Vallota, and H. J. Müller-Eberhard, in preparation). This so called C3 nephritic factor(s) which persists after nephrectomy is now thought to be an activated normal constituent of the initial stages of the alternate pathway, reacting before properdin [61]. C3 depression without concomitant depression of initial C components (C1, C4, C2) [62], as well as decreased properdin [63] and C3 proactivator [64] concentrations, suggest utilization of the alternate pathway and is typical of childhood hypocomplementemic glomerulonephritis and, to a lesser extent, acute poststreptococcal glomerulonephritis. Utilization of the entire C sequence in addition to properdin is seen in SLE and indicates classical C activation with possible additional alternate C pathway activation.

The serum observations can be extended to immunofluorescence detection of C components in glomeruli. The presumed immune complex glomerulonephritides, as identified by granular Ig deposits, are almost always associated with granular C deposits [65]. Both initial and late acting components are usually present, indicating classical activation has occurred. In anti-GBM antibody nephritis, C deposits are found in 65 to 75% of patients; and in many of these patients the deposits are less striking than the Ig deposits and are usually interrupted and irregular rather than linear. When C3 is present, both early and late acting C components can usually be identified. In one-third to one-fourth of patients who have few or no C deposits, other as yet unidentified pathways must be responsible for the antibody-induced injury. No obvious differences in the severity of the clinical courses of patients with anti-GBM nephritis have been related to the presence or absence of C deposits in the few patients thus observed [65].

In about 10% of renal tissue studied (usually from patients in advanced stages of glomerular injury), C3 (Table 1) and later acting C components may be present in the absence of Ig and initial C components [65]. Properdin may also be detected [66]. Similar findings have been observed in childhood hypocomplementemic glomerulonephritis, but even then the majority of patients have Ig and initial C components sometime during the course of their disease [67]. Little is known about the relative disappearance rates of Ig and C from glomerulonephritic kidneys, but limited serial observations suggest that C may persist or even increase while Ig becomes undetectable. Indeed, we have observed kidneys in which Ig and both initial and late C components are present in a minority of glomeruli, while only intense C3 and later C components are found in the majority of glomeruli [65]. This suggests a transition in pathogenesis is occurring, with perpetuation of a preceding immune complex-induced glomerular injury through an as yet unexplained continuing C deposition possibly utilizing the alternate C pathway.

Tubular and interstitial injury. Interest is beginning to focus on immune processes which may produce tubular and interstitial injury, either associated with glomerulonephritis or occurring as a primary disease [68, 69]. Seventy percent of patients with anti-GBM antibody-induced nephritis have evidence of anti-TBM antibodies as well. The reactivity of the anti-TBM antibodies is usually restricted to a portion of the tubules, but occasionally may be more generalized. Infrequently, anti-TBM antibodies without associated anti-GBM reactivity can be identified in allografted kidneys [70] and in association with methicillin toxicity (W. A. Border, D. H. Lehman, J. D. Egan and C. B. Wilson, in preparation).

Granular deposits of Ig and C which suggest immune complexes are found in the TBM, peritubular capillaries and/or interstitium of almost 75% of SLE kidneys. Similar extraglomerular deposits are in-

frequent in other immune complex glomerulonephritides (D. H. Lehman and C. B. Wilson, in preparation). Many kidneys, occasionally essentially normal ones, have tubular and vascular deposits of C3 in the absence of Ig.

Granular immunoglobulin deposits in and about the TBM, peritubular capillaries and in the interstitium are presumed to represent immune complex deposits. In experimental serum sickness it has been possible to demonstrate antibody, antigen and C in similar locations, indicating that they are probably combined as immune complexes. Granular deposits of unknown origin and significance are also observed in renal tubular cells, and are often associated with the TBM in rabbits immunized with renal tubular antigens. Electron microscopic localization of these deposits between the renal tubular plasma membrane and the TBM suggests that they may represent *in situ* formed immune complexes, similar to those seen in experimental and clinical thyroiditis.

Immunopathologic studies of renal transplants. Immune injury to renal transplants may occur acutely within minutes of transplantation or over prolonged periods of time [71]. Hyperacute rejection is induced by preformed antibodies which can sometimes be demonstrated by immunofluorescence as being diffusely bound to the vascular endothelium [72]. Tissue injury is mediated at least in part by polymorphonuclear leukocytes and coagulation, with FRA depositing heavily in glomeruli and vessels. The amount of fibrin present often makes it difficult to be certain that accompanying Ig and C deposits have immunologic specificity, since they may merely be trapped within a fibrin coagulum. Quantities of antibody sufficient to induce hyperacute rejection may also never reach the concentration necessary for immunofluorescence detection.

The same immunopathologic techniques utilized to identify the immune processes responsible for primary disease are applicable to the study of more chronic immune injury in renal transplants. Interpretation of the findings is complicated by the multiple events which may take place simultaneously in the transplant. First, the transplanted kidney is subjected to the same immune processes which were responsible for the patient's original renal disease. Second, allografts are exposed to varied immune responses generated by the rejection process. Also, the allograft can potentially induce immune responses to renal constituents not usually histoincompatible, such as basement membranes and renal tubular antigens, thus stimulating production of potentially nephrotoxic anti-basement membrane antibodies and circulating immune complexes. Third, administration of various immunosuppressive regimens, including foreign serum proteins in the form of antilymphocyte sera, almost surely modifies the contributions of the other two major factors.

Recurrence of glomerulonephritis in renal transplants has been clearly shown in transplants in rats with autologous immune complex glomerulonephritis [73] and in unmodified renal transplants from identical human twins [74]. In this latter instance, glomerulonephritis recurred in 11 of 18 patients within six years of transplantation, while similar lesions were not observed in nonglomerulonephritic patients in the same series. Anti-GBM antibodies can also cause recurrent glomerulonephritis, particularly when they are present in the circulation at time of transplantation [2, 75, 76]. Immunofluorescence and elution studies can confirm the presence of anti-GBM antibodies in the transplant. To complicate matters, anti-GBM (C. B. Wilson, unpublished observations) and anti-TBM antibodies [70] related to immunologic stimuli from the grafts may develop in transplanted patients. Adequate pre- and posttransplant studies are required to document recurrence of anti-GBM nephritis.

Clinical, morphologic and/or immunofluorescence studies strongly suggest recurrence of "IgA-IgG nephritis" [30], idiopathic nephrotic syndrome [77], dense deposit disease [78] and hypocomplementemic glomerulonephritis [79] in renal transplant patients. That immune complex-induced glomerular injury can recur in transplants (as demonstrated by immunofluorescence and morphologic observations) can scarcely be doubted; however, a true recurrence cannot be confirmed until identical antigen-antibody systems can be identified in both the native and transplanted kidneys. Induction of autologous immune complex nephritis by implantation of unvascularized kidneys in the peritoneal cavity of rats [80] and induction of nephritis in graft *vs.* host reactions in which H2 antigens may be involved in immune complex formation [81] indicates that antigens within a renal transplant are potentially capable of inducing immune complex nephritis. Only when techniques are developed to detect the antigenic component of immune complexes will these complicated problems be solved.

Renal allografts that are rejected within the first few days may have few or no detectable Ig or C glomerular deposits, and vascular deposits of Ig, particularly IgM, C and FRA vary. Long-term renal allografts frequently have irregular glomerular deposits of Ig and C deposits often similar to those seen in patients with immune complex glomerulonephritis [82–84]. Vascular deposits of Ig and C are also frequent. Since similar deposits are occasionally observed in patients without previous glomerulone-

phritis, it is apparent that, in some instances, these deposits are related to immune processes directed against the graft rather than to recurrence of a nephritic process. In our experience, Ig and C deposits in glomeruli of transplants are most frequent in patients with a previous glomerulonephritis. These observations are similar to those made in identical twins. When glomerular deposits do occur in non-nephritic transplanted patients, their occurrence seems to be related in part to the degree of histoincompatibility (as currently detected) between the graft and the recipient, with patients thought to have HLA-identical matches having fewer deposits. Only greatly expanded pre- and posttransplant studies and advances in immune complex antigen identification can determine the relative frequencies of recurrent nephritis and rejection-related immune injury. The potential development of serum sickness and antibasement membrane nephritis [85] must also be kept in mind when transplant recipients are receiving antilymphocyte globulin preparations.

Conclusions. The mechanisms and diagnostic features of immunologic renal damage have been outlined. Identification of the nephritogenic GBM antigen(s) and the events responsible for induction of the "autoimmune" anti-GBM response are being studied. Many investigators are searching for the antigen-antibody systems which could be responsible for the many presumed immune complex human glomerulonephritides identified now only by their granular glomerular Ig and C deposits. To aid in this search, physicians, by being alert to potential immune responses in individual patients, can narrow the almost limitless antigenic possibilities to a few testable systems. The existence of alternate and possibly non-immune complement activation suggests a possible additional mechanism of glomerular injury. It is also becoming increasingly clear that immune mechanisms similar to those producing glomerular injury may be involved in tubular and interstitial injury; this points out the need for correlative immunopathologic, structural and functional studies of tubules.

CURTIS B. WILSON
FRANK J. DIXON
La Jolla, California

This is publication no. 799 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California. The work was supported in part by USPHS contract 1-AI-42505 and USPHS grant AI-07007.

Reprint requests to Dr. C. B. Wilson, Dept. of Experimental Pathology, Scripps Clinic and Research Foundation, 476 Prospect St., La Jolla, California 92037, U.S.A.

References

1. AGNELLO V, KOFFLER D, KUNKEL HG: Immune complex systems in the nephritis of systemic lupus erythematosus. *Kidney Int* 3:90–99, 1973
2. WILSON CB, DIXON FJ: Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int* 3: 74–89, 1973
3. BALCH CM, WILSON CB, LEE S, FELDMAN JD: Thymus-dependent lymphocytes in tissue sections of rejecting rat renal allografts. *J Exp Med* 138:1584–1590, 1973
4. PLOEM JS: The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescence microscopy with incident light. *Mikrosk Technik* 68:129–142, 1967
5. MARKHAM RV, SUTHERLAND JC, MARDINEY MR: The ubiquitous occurrence of immune complex localization in the renal glomeruli of normal mice. *Lab invest* 29:111–120, 1973
6. GLENNES GC: Immunoglobulin and amyloid fibril proteins. *Br J Haematol* 24:533–537, 1973
7. WOOD BT, THOMPSON SH, GOLDSTEIN G: Fluorescent antibody straining: III. Preparation of fluorescein-isothiocyanate-labeled antibodies. *J Immunol* 95:225–229, 1965
8. EDGINGTON TS: Dissociation of antibody from erythrocyte surfaces by chaotropic ions. *J Immunol* 106:673–680, 1971
9. KOFFLER D, AGNELLO V, CARR RI, KUNKEL HG: Variable patterns of immunoglobulin and complement deposition in the kidneys of patients with systemic lupus erythematosus. *Am J Pathol* 56:305–316, 1969
10. WESTBERG NG, MICHAEL AF: Immunopathology of diabetic glomerulonephritis. *Diabetes* 2:163–174, 1972
11. MARQUARDT H, WILSON CB, DIXON FJ: Isolation and immunological characterization of human glomerular basement membrane antigens. *Kidney Int* 3:57–65, 1973
12. KUPOR LR, MCPHAUL JJ: Mechanisms of idiopathic renal hematuria (abstract). *Am Soc Nephrol*, 1973, p. 62
13. MAHIEU P, DARDENNE M, BACH JF: Detection of humoral and cell-mediated immunity to kidney basement membranes in human renal diseases. *Am J Med* 53:185–192, 1972
14. MACANOUIC M, EVANS DJ, PETERS DK: Allergic response to glomerular basement membrane in patients with glomerulonephritis. *Lancet* 2:207–210, 1972
15. MAHIEU PM, LAMBERT PH, MAGHUIN-ROGISTER GR: Primary structure of a small glycoprotein isolated from human glomerular basement membrane and carrying a major antigenic site *Eur J Biochem* 40:599–606, 1973
16. KLASSEN J, ELWOOD C, GROSSBERG A, MILGROM F, MONTES M, SEPULVEDA M, ANDRES G: Evolution of an immune complex glomerulonephritis into rapidly progressive anti-glomerular basement membrane glomerulonephritis (abstract). *Am Soc Nephrol*, 1973, p. 59
17. ROCKLIN RE, LEWIS EJ, DAVID JR: *In vitro* evidence for cellular hypersensitivity to glomerular-basement-membrane antigens in human glomerulonephritis. *N Engl J Med* 283: 497–501, 1970
18. GOTOFF SP, QUARASHI MM, MALECKI TJ: Cellular response to glomerular-basement-membrane antigen. *Lancet* 2:763–764, 1972
19. LEWIS EJ, BUSCH GJ, SCHUR PH: Gamma G globulin subgroup composition of the glomerular deposits in human renal diseases. *J Clin Invest* 49:1103–1113, 1970
20. MCPHAUL JJ JR, DIXON FJ: Characterization of immunoglobulin G anti-glomerular basement membrane antibodies eluted from kidneys of patients with glomerulonephritis: II.

IgG subtypes and *in vitro* complement fixation. *J Immunol* 107:618–684, 1971

21. Beirne GJ, Brennan JT: Glomerulonephritis associated with hydrocarbon solvents: Mediated by antiglomerular basement membrane antibody. *Arch Environ Health* 25: 365–369, 1972
22. Wilson CB, Smith RC: Goodpasture's syndrome associated with influenza A2 virus infection. *Ann Intern Med* 76:91–94, 1972
23. Beirne GJ, Kopp WL, Zimmerman SW: Goodpastures syndrome: Dissociation from antibodies to glomerular basement membrane. *Arch Intern Med* 132:261–263, 1973
24. Lewis EJ, Schur PH, Busch GJ, Galvanek E, Merrill JP: Immunopathologic features of a patient with glomerulonephritis and pulmonary hemorrhage. *Am J Med* 54:507–513, 1973
25. Mauer SM, Sutherland ER, Howard RJ, Fish AJ, Najarian JS, Michael AF: The glomerular mesangium: III. Acute immune mesangial injury: A new model of glomerulonephritis. *J Exp Med* 137:553–570, 1973
26. Roy LP, Westberg NG, Michael AF: Nephrotic syndrome—no evidence for a role for IgE. *Clin Exp Immunol* 13:553–559, 1973
27. Lewis EJ, Kallen RJ, Rowe DS: Glomerular localization of IgE in lipoid nephrosis. *Lancet* 1:1395, 1973
28. Urizan RE, Herdman RC: Anaphylactoid purpura: III. Early morphologic glomerular changes. *Am J Clin Pathol* 53: 258–266, 1970
29. Evans DJ, Williams DG, Petero DK, Sissons JGP, Boulton-Jones JM, Ogg CS, Cameron JS, Hoffbrand BI: Glomerular deposition of properdin in Henoch-Schonlein Syndrome and idiopathic focal nephritis. *Br Med J* 3:326–328, 1973
30. Berger J: IgA glomerular deposits in renal disease. *Transplant Proc* 1:939–944, 1969
31. Roy PL, Fish AJ, Vernier RL, Michael AF: Recurrent macroscopic hematuria, focal nephritis, and mesangial deposition of immunoglobulin and complement. *J Pediatr* 82:767–772, 1973
32. Lowance DC, Mullins JD, McPhaul JJ: Immunoglobulin A (IgA) associated glomerulonephritis. *Kidney Int* 3: 167–176, 1973
33. Hyman LR, Wagnild JP, Beirne GJ, Burkholder PM: Immunoglobulin—A distribution in glomerular disease: Analysis of immunofluorescence localization and pathogenetic significance. *Kidney Int* 3:397–408, 1973
34. Mowbray JF, Hoffbrand AV, Holborow EJ, Seah PP, Fry L: Circulating immune complexes in dermatitis herpetiformis. *Lancet* 1:400–402, 1973
35. Theofilopoulos AN, Bokisch VA, Dixon FJ: The use of Raji cells for the detection of circulating complexes in experimental animals and humans. *Fed Proc*, in press
36. Wilson CB, Dixon FJ, Evans AS, Glassock RJ: Antiviral antibody responses in patients with renal disease. *Clin Immunol Immunopathol* 2:121–132, 1973
37. Fiala M, Guze LB, Goldberg M, Henderson LW, Glassock RJ, Parsa KP: Cultures of renal biopsies: Outgrowth of vacuolated and multinucleated cells. *Nephron* 11:276–286, 1973
38. Bariety J, Richer D, Appay MD, Grossetete J, Callard P: Frequency of intraendothelial "virus like" particles: An electron microscopy study of 376 human renal biopsies. *J Clin Pathol* 26:21–24, 1973
39. Gocke DJ, Hsu K, Morgan C, Bombardieri S, Lockshin M, Christian CL: Association between polyarteritis and Australia antigen. *Lancet* 2:1149–1153, 1970
40. Trepo CH, Thivolet J, Prince AM: Au-SH antigen, hepatitis, and polyarteritis nodosa. *Prog Immunobiol Stand* 5:99–103, 1972
41. Combes B, Stastny P, Shorey J, Eigenbrodt EH, Barrera A, Hall AR, Carter NW: Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane. *Lancet* 2:234–237, 1971
42. Fauci AS, Wolff SM: Wegener's granulomatosis: Studies in eighteen patients and a review of the literature. *Medicine (Baltimore)* 52:535–561, 1973
43. McGiven AR, de Boer WGRM, Barnett AJ: Renal immune deposits in scleroderma. *Pathology* 3:145–150, 1971
44. Burkholder PM: Malignant nephrosclerosis: An immunohistopathologic study on localized γ-globulin and fixation of guinea pig complement in human kidneys. *Arch Pathol* 80: 583–589, 1965
45. Paronetto F: Immunocytochemical observations on the vascular necrosis and renal glomerular lesions of malignant nephrosclerosis. *Am J Pathol* 46:901–915, 1965
46. Lieberman E: Hemolytic-uremic syndrome. *J Pediatr* 80: 1–16, 1972
47. Gianatonio CA, Vitacco M, Mendilaharzu F, Gallo GE, Sojo ET: The hemolytic-uremic syndrome. *Nephron* 11: 174–192, 1973
48. Kaufman DB, McIntosh RM, Smith FG Jr, Vernier RL: Diffuse familial nephropathy: A clinicopathological study. *J Pediatr* 77:37–47, 1970
49. Spear GS, Slusser RJ: Alport's syndrome: Emphasizing electron microscopic studies of the glomerulus. *Am J Pathol* 69:313–224, 1972
50. Mauer SM, Michael AF, Fish AJ, Brown DM: Spontaneous immunoglobulin and complement deposition in glomeruli of diabetic rats. *Lab Invest* 27:488–494, 1972
51. Mauer SM, Sutherland DER, Steffes MW, Leonard RJ, Najarian JS, Michael AF, Brown DM: Effects of pancreatic transplantation on the glomerular lesions of experimental diabetes in the rat. *Clin Res* 21:895, 1973
52. Arisz L, Brentjens RH, van der Hem GK, Mandema E: Clinical value of serum B_{1A} and B_{1E} globulin levels in adult patients with renal disease. *Acta Med Scand* 192:255–261, 1972
53. Gewurz H, Pickering RJ, Mergenhogen SE, Good RA: The complement profile in acute glomerulonephritis, systemic lupus erythematosus and hypocomplementemic chronic glomerulonephritis. *Int Arch Allergy Appl Immunol* 34:556–570, 1968
54. Cameron JS, Vick RM, Ogg CS, Seymour WM, Chantler C, Turner DR: Plasma C3 and C4 concentrations in management of glomerulonephritis. *Br Med J* 3:668–672, 1973
55. Götze O, Müller-Eberhard HJ: The role of properdin in the alternate pathway of complement activation. *J Exp Med*, in press
56. Götze O, Müller-Eberhard HJ: The C3-activator system: An alternate pathway of complement activation. *J Exp Med* 134:91s–108s, 1971
57. Ruley EJ, Forristal J, Davis NC, Andres G, West CD: Hypocomplementemia of membranoproliferative nephritis: Dependence of the nephritic factor reaction on properdin factor B. *J Clin Invest* 52:896–904, 1973

58. MÜLLER-EBERHARD HJ, GÖTZE O: C3 proactivator convertase and its mode of action. *J Exp Med* 135:1003–1008, 1972
59. WILLIAMS DG, CHARLESWORTH JA, LACHMANN PJ, PETERS DK: Role of C3b in the breakdown of C3 in hypocomplementaemic mesangiocapillary glomerulonephritis. *Lancet* 1: 447–449, 1973
60. VALLOTA EH, FORRISTAL J, SPITZER RE, DAVIS NC, WEST CD: Characteristics of a non-complement-dependent C3-reactive complex formed from factors in nephritic and normal serum. *J Exp Med* 131:1306–1324, 1970
61. VALLOTA EH, GÖTZE O, SPIEGELBERG HL, FORRISTAL J, WEST CD, MÜLLER-EBERHARD HJ: A serum factor in chronic hypocomplementemic nephritis distinct from immunoglobulin and activating the alternate pathway of complement. *J Exp Med*, in press
62. WEST CD, RULEY EJ, FORRISTAL J, DAVIS NC: Mechanisms of hypocomplementemia in glomerulonephritis. *Kidney Int* 3:116–125, 1973
63. MCLEAN RH, MICHAEL AF: Properdin and C3 proactivator: Alternate pathway components in human glomerulonephritis. *J Clin Invest* 52:634–644, 1973
64. PERRIN LH, LAMBERT PH, NYDEGGER UE, MIESCHER PA: Quantitation of C3PA (Properdin Factor B) and other complement components in diseases associated with a low C3 level. *Clin Immunol Immunopathol* 2:16–27, 1973
65. VERROUST PJ, WILSON CB, COOPER NR, EDGINGTON TS, DIXON FJ: Glomerular complement components in human glomerulonephritis. *J Clin Invest* 53:77–84, 1974
66. WESTBERG NG, NAFF GB, BOYER JT, MICHAEL AF: Glomerular deposition of properdin in acute and chronic glomerulonephritis with hypocomplementemia. *J Clin Invest* 50:642–649, 1971
67. MICHAEL AF, WESTBERG NG, FISH AJ, VERNIER RL: Studies on chronic membranoproliferative glomerulonephritis with hypocomplementemia. *J Exp Med* 134:208s–227s, 1971
68. KLASSEN J, ANDRES GA, BRENNAN JC, MCCLUSKEY RT: An immunologic renal tubular lesion in man. *Clin Immunol Immunopathol* 1:69–83, 1972
69. MCCLUSKEY RT, KLASSEN J: Immunologically mediated glomerular, tubular and interstitial renal disease. *N Engl J Med* 288:564–570, 1973
70. KLASSEN J, KANO K, MILGROM F, MENNO AB, ANTHONE S, ANTHONE R, SEPULVEDA M, ELWOOD CM, ANDRES GA: Tubular lesions produced by autoantibodies to tubular basement membrane in human renal allografts. *Int Arch Allergy Appl Immunol* 45:675–689, 1973
71. PORTER KA: Clinical renal transplantation. *Int Rev Exp Pathol* 11:73–176, 1972
72. WILLIAMS GM, HUME DM, PAGE HUDSON R JR, MORRIS PJ, KANO K, MILGROM F: "Hyperacute" renal-homograft rejection in man. *N Engl J Med* 279:611–618, 1968
73. EDGINGTON TS, LEE S, DIXON FJ: Persistence of the autoimmune pathogenetic process in experimental autologous immune complex nephritis. *J Immunol* 103:528–536, 1969
74. GLASSOCK RJ, FELDMAN D, REYNOLDS ES, DAMMIN GJ, MERRILL JP: Human renal isografts: A clinical and pathologic analysis. *Medicine (Baltimore)* 47:411–454, 1968
75. LERNER RA, GLASSOCK RJ, DIXON FJ: The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med* 126:989–1004, 1967
76. MCPHAUL JJ JR, THOMPSON AL JR, LORDON RE, KLEBANOFF G, COSIMI AB, DELEMOS R, SMITH RB: Evidence suggesting persistance of nephritogenic immunopathologic mechanisms in patients receiving renal allografts. *J Clin Invest* 52:1059–1066, 1973
77. HOYER JR, RAIJ L, VERNIER RL, SIMMONS RL, NAJARIAN JS, MICHAEL AF: Recurrence of idiopathic nephrotic syndrome after renal transplantation. *Lancet* 2:343–348, 1972
78. GALLE P, HINGLAIS N, CROSNIER J: Recurrence of an original glomerular lesion in three renal allografts. *Transplant Proc* 3:368–370, 1971
79. MCLEAN RH, GEIGER H, SIMMONS R, NAJARIAN J, MICHAEL AF: Recurrence of membranoproliferative glomerulonephritis (MPGN) following kidney transplantation (abstract). *Am Soc Nephrol*, 1973, p. 70
80. MILGROM F, KLASSEN J, KANO K: Autoimmunity and homograft rejection. *J Reticuloendothel Soc* 7:264–279, 1970
81. KANO K, BELDOTTI L, MILGROM F, SCHWARTZ RS: Glomerulonephritis in the graft-vs-host reaction: Serologic demonstration of anti-host antibodies. *J Immunol* 112: 410–411, 1974
82. MCPHAUL JJ JR, DIXON FJ, BRETTSCHNEIDER L, STARZL TE: Immunofluorescent examination of biopsies from long-term renal allografts. *N Engl J Med* 282:412–417, 1970
83. ANDRES GA, ACCINNI L, HSU KC, PENN I, PORTER KA, RENDALL JM, SEEGAL BC, STARZL TE: Human renal transplants: III. Immunopathologic studies. *Lab Invest* 22: 588–604, 1970
84. BUSCH GJ, GALVANEK EG, REYNOLDS ES JR: Human renal allografts. Analysis of lesions in long-term survivors. *Hum Pathol* 2:253–298, 1971
85. WILSON CB, DIXON FJ, FORTNER JG, CERILLI J: Glomerular basement membrane reactive antibodies in anti-lymphocyte globulin. *J Clin Invest* 50:1525–1535, 1971
86. FISH AJ, HERDMAN RC, MICHAEL AF, PICKERING RJ, GOOD RA: Epidemic acute glomerulonephritis associated with type 49 streptococcal pyoderma. *Am J Med* 48:28–39, 1970
87. MOREL-MAROGER L, LEATHEM A, RICHET G: Glomerular abnormalities in nonsystemic diseases: Relationship between findings by light microscopy and immunofluorescence in 433 renal biopsy specimens. *Am J Med* 53:170–184, 1972
88. LEWIS EJ, CAVALLO T, HARRINGTON JT, COTRAN RS: An immunopathologic study of rapidly progressive glomerulonephritis in the adult. *Hum Pathol* 2:185–208, 1971
89. GERMUTH FG, RODRIGUEZ E: Immunopathology of the renal glomerulus: *Immune Complex Deposit and Anti-basement Membrane Disease*. Boston, Little, Brown and Company, 1973
90. HYMAN LR, BURKHOLDER PM: Focal sclerosing glomerulonephropathy with segmental hyalinosis: A clinicopathologic analysis. *Lab Invest* 28:533–544, 1973
91. COCHRANE CG, KOFFLER D: Immune complex disease in experimental animals and man. *Adv Immunol* 16:186–264, 1973